



Manual of Procedures

**National Centralized Repository for Alzheimer's Disease and Related
Dementias (NCRAD)**

**A Phase 2 Randomized Double-Blind Placebo-Controlled Trial to
Evaluate the Efficacy and Safety of BHV-4157 in Patients with Mild to
Moderate Alzheimer's Disease (T2 PROTECT AD)**

Biospecimen Collection, Processing, and Shipment Manual

Version 02.2020

Table of Contents

1.0	Abbreviations	4
2.0	Purpose	4
3.0	NCRAD Information	5
3.1	NCRAD Contacts	
3.2	Hours of Operation	
3.3	Holiday Schedules	
3.4	Holiday Observations	
4.0	Globally Unique Identifier (GUID)	6
5.0	NCRAD Laboratory Collection	7
5.1	Site Required Equipment	
5.2	Biospecimens Sent to NCRAD	
5.3	Biospecimen Collection Charts	
6.0	Specimen Collection Kits, Shipping Kits, and Supplies	12
6.1	Specimen Collection Kit Contents	
6.2	Kit Supply to Study Sites	
7.0	Blood Collection and Processing Procedures	16
7.1	Labeling Samples	
7.2	Video List	
7.3	Filling Aliquot Tubes (Plasma and Serum)	
7.4	Serum (Red-Top) Tube (6 ml) for Serum	
7.5	EDTA (Lavender-Top) Tube (6 mL) for PK Analysis	
7.6	EDTA (Lavender-Top) Tube (10 ml) for Plasma and Buffy Coat	
8.0	Cerebrospinal Fluid Collection	33
8.1	Scheduling the LP	
8.2	Performing the LP	
9.0	Sample Redraws	42
10.0	Packaging and Shipping Instructions	43
10.1	Frozen Shipping Instructions	
11.0	Data and Query Reconciliation	47



12.0	<u>Appendices List</u>	48
12.1	<u>Appendix A: Rate of Centrifugation Worksheet</u>	
12.2	<u>Appendix B: Biological Sample and Shipment Notification Form</u>	
12.3	<u>Appendix C: CSF Sample and Shipment Notification Form</u>	
12.4	<u>Appendix D: GUID Demographics Form</u>	

1.0 Abbreviations

AD	Alzheimer's Disease
ADCS	Alzheimer's Disease Cooperative Study
BL	Baseline Visit
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protective Health Information
RBCs	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide T2 PROTECT AD staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for T2 PROTECT AD study visits. It includes instructions for biospecimen submission to the National Cell Repository for Alzheimer's Disease (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)
- CSF

This manual includes instructions for collection of blood and CSF, fractionation of blood from collection tubes, aliquoting, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to NCRAD for the T2 PROTECT AD protocols.



3.0 NCRAD Information

3.1 NCRAD Contacts

Kelly Nudelman, PhD, Core Leader

Phone: 317-963-7511

Email: kholohan@iu.edu

Kelley Faber, MS, CCRC, Project Manager

Phone: 317-274-7360

Email: kelfaber@iu.edu

General NCRAD Contact Information

Phone: 1-800-526-2839

Fax: 317-321-2003

Email: alzstudy@iu.edu

Website: www.ncrad.org

T2 PROTECT AD Study Specific Webpage: https://ncrad.org/resource_t2.html

Milena Petkov, BS, CCRP, Study Coordinator

Phone: 317-278-1228

Email: mipetkov@iu.edu

Sample Shipment Mailing Address

NCRAD

Indiana University School of Medicine

351 W. 10th St

TK-217

Indianapolis, IN 46202

3.2 Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped **Monday-Wednesday only**.

Check weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not affect the shipping or delivery of the samples.

3.3 Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.

3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week of December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples.

Please see: https://ncrad.org/holiday_closures.html for additional information.

4.0 Globally Unique Identifier (GUID)

The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies.

To create a GUID follow these steps:

1. Create an account: <https://bricsguid.nia.nih.gov/portal/jsp/login.jsp>
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the ‘Launch GUID Tool’ you will need to have Java installed on your device
4. In order to generate a GUID, the following PHI is required ([Appendix D](#)):
 - Complete legal given (first) name of subject at birth
 - If the subject has a middle name
 - Complete legal family (last) name of subject at birth
 - Day of birth
 - Month of birth
 - Year of birth
 - Name of city/municipality in which subject was born
 - Country of birth

5.0 NCRAD Laboratory Information

5.1 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket
- Wet ice
- Dry ice

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- Centrifuge capable of $\geq 2000 \times g$ with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice (about approximately 30-45 lbs per shipment)

5.2 Biospecimens Sent to NCRAD

Biospecimens collected include whole blood and CSF. Please refer to the below schedule for the biospecimen collection schedule:

	Screening	Baseline	Week 4	Week 8	Week 12	Week 24	Week 48
Serum		X				X	X
Plasma		X					X
Buffy Coat		X					X
PK		X	X	X	X	X	X
CSF	X*					X	X

*Screening LP to be completed between screening and baseline visits

Whole blood is collected in two different collection tubes: lavender-top EDTA tubes and plain red-top serum tubes. At Baseline and Week 48 visits, the lavender-top EDTA tube is processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. At Baseline, Week 24, and Week 48, the plain red-top serum tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. Blood for PK analysis is collected at Baseline, Weeks 4, 8, 12, 24, and 48, processed locally and aliquots are shipped frozen to NCRAD.

CSF will be aliquoted locally, frozen at the study site, and then shipped to NCRAD.

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. A copy of the consent form for each subject should be kept on file by the site investigator.

Frozen samples are to be submitted according to the shipping methods outlined in [Section 10.2](#). Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

5.3 Biospecimen Collection Charts

5.3.1 Biospecimen Collection for Screening Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
CSF	Sterile Containers (15 ml CSF)	30	CSF: 0.5 ml CSF aliquots per 2.0 ml orange cryovial	Up to 30	Frozen

5.3.2 Biospecimen Collection for Baseline Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (6 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	2	SERUM: 1.5 ml serum aliquots per 2.0 ml cryovial	2	Frozen
Whole blood for isolation of plasma-pk	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	N/A	N/A	N/A
	Plasma-PK: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	6	PLASMA: 0.5 ml plasma aliquots per 2.0 ml cryovial	Up to 6	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	PLASMA: 1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 4	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen

5.3.3 Biospecimen Collection for Week 4, 8, and 12 Visits

Sample Type	Collection Tube	Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for PK analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	N/A	N/A	N/A
	Plasma PK: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	6	0.5 mL aliquots per 2.0 mL cryovials	Up to 6	Frozen

5.3.4 Biospecimen Collection for Week 24 Visit

Sample Type	Collection Tube	Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (6 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	2	SERUM: 1.5 ml serum aliquots per 2.0 ml cryovial	2	Frozen
Whole blood for PK analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	N/A	N/A	N/A
	Plasma PK: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	6	0.5 mL aliquots per 2.0 mL cryovials	Up to 6	Frozen
CSF	Sterile Containers (15 ml CSF)	30	CSF: 0.5 ml CSF aliquots per 2.0 ml orange cryovial	Up to 30	Frozen

5.3.5 Biospecimen Collection for Week 48 Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (6 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	2	SERUM: 1.5 ml serum aliquots per 2.0 ml cryovial	2	Frozen
Whole blood for PK analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	N/A	N/A	N/A
	Plasma PK: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	6	0.5 mL aliquots per 2.0 mL cryovials	Up to 6	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	PLASMA: 1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 4	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen
CSF	Sterile Containers (15 ml CSF)	30	CSF: 0.5 ml CSF aliquots per 2.0 ml orange cryovial	Up to 30	Frozen

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form** (see [Appendix B](#)). Submit a copy to NCRAD with a reason provided for the omission.

6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, and the Frozen Shipment Kit; 2) CSF collection kits including Lumbar Puncture (LP) trays, and the CSF Supplemental Supply Kit; 3) clinical lab supplies (with the exception of dry ice and equipment supplies listed in [Section 5.1](#)). These materials include blood tubes, pipettes, LP trays (when applicable), boxes for serum/plasma/buffy coat/PK/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and ADCS ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood and CSF Kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to [Section 7.1](#).

6.1 Specimen Collection Kit Contents

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NCRAD Study team to do so. Please store all kits at room temperature until use.

T2 PROTECT AD Blood Collection Kit-Baseline

Quantity	Blood Collection Kit Components
1	Serum Red Top Blood Collection Tube (6 mL)
1	EDTA Lavender Top Blood Collection Tube (6 mL)
1	EDTA Lavender Top Blood Collection Tube (10 mL)
9	Cryovial tube (2.0 mL) with lavender cap
1	Cryovial tube (2.0 mL) with clear cap
2	Cryovial tube (2.0 mL) with red cap
3	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette
18	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
4	Labels for Handwritten Site and ADCS ID
1	Microcentrifuge box (25-slot)

T2 PROTECT AD Blood Collection Kit- Weeks 4, 8, and 12

Quantity	Blood Collection Kit Components
1	EDTA Lavender Top Blood Collection Tube (6 mL)
6	Cryovial tube (2.0 mL) with lavender cap

1	Cryovial tube (2.0 mL) with blue cap
8	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
2	Label for Handwritten Site and ADCS ID
1	Microcentrifuge box (25-slot)

T2 PROTECT AD Blood Collection Kit- Week 24

Quantity	Blood Collection Kit Components
1	Serum Red Top Blood Collection Tube (6 mL)
1	EDTA Lavender Top Blood Collection Tube (6 mL)
2	Cryovial tube (2.0 mL) with red cap
6	Cryovial tube (2.0 mL) with lavender cap
2	Cryovial tube (2.0 mL) with blue cap
12	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
3	Label for Handwritten Site and ADCS ID
1	Microcentrifuge box (25-slot)

T2 PROTECT AD Blood Collection Kit- Week 48

Quantity	Blood Collection Kit Components
1	Serum Red Top Blood Collection Tube (6 mL)
1	EDTA Lavender Top Blood Collection Tube (10 mL)
1	EDTA Lavender Top Blood Collection Tube (6 mL)
9	Cryovial tube (2.0 mL) with lavender cap
1	Cryovial tube (2.0 mL) with clear cap
2	Cryovial tube (2.0 mL) with red cap
3	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette
18	Pre-printed Collection and Aliquot Tube Label
3	Pre-printed Kit Number Label
3	Labels for Handwritten Site and ADCS ID
1	Microcentrifuge box (25-slot)

Blood Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
5	Serum (Red-Top) Blood Collection Tube (6 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
10	Cryovial tube (2.0 ml) with lavender cap
10	Cryovial tube (2.0 ml) with red cap
10	Cryovial tube (2.0 ml) with blue cap
5	Cryovial tube (2.0 ml) with clear cap

20	Disposable graduated transfer pipette
10	Labels for handwritten Site and ADCS ID
5	Microcentrifuge box (25-slot)

T2 PROTECT AD Frozen Shipping Kit

Quantity	Frozen Shipping Kit Components
5	Plastic Biohazard bag with absorbent sheet (small)
1	FedEx return airbill and pouch
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

T2 PROTECT AD LP Kits

***Sites must specify 22 or 24 gauge kit when ordering from NCRAD.**

Quantity	LP Kit Components
1	Sprotte needle, 22 or 24 gauge X 3.5" (90mm)
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22 gauge x 1.5"
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8" needle attached
4	Polypropylene syringe (5 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18" x 26"
2	Towel, 13.5" x 18"
6	Gauze pad, 2" x 2"
3	Sponge stick applicator
2	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

T2 PROTECT AD CSF Kits

Quantity	CSF Supplemental Supply Kit Components
2	15-ml conical polypropylene tube-individually wrapped
30	Cryovial tube (2.0 ml) with orange cap
1	Cryovial tube (2.0 ml) with blue cap
1	Cryovial tube (2.0 ml) with yellow cap
2	Disposable pipet
1	Microcentrifuge box (25-slot)

Supplemental CSF Kits

Quantity	CSF Supplemental Supply Kit Components
20	15-ml conical polypropylene tube-individually wrapped
50	Cryovial tube (2.0 ml) with orange cap
5	Cryovial tube (2.0 ml) with blue cap
5	Cryovial tube (2.0 ml) with yellow cap
5	3 ½" × 22 or 24G Sprotte needle with Introducer (90mm)

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	Microcentrifuge box (25-slot)
By Request	Cryovial tube (2.0 ml) with lavender cap
By Request	Cryovial tube (2.0 ml) with red cap
By Request	Cryovial tube (2.0 ml) with orange cap
By Request	Cryovial tube (2.0 ml) with yellow cap
By Request	Cryovial tube (2.0 ml) with blue cap
By Request	Cryovial tube (2.0 ml) with clear cap
By Request	15-ml conical polypropylene tube-individually wrapped
By Request	FedEx return airbill
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette
By Request	Plain Red Top Serum (Red-Top) Blood Collection Tube (6 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	UN3373 label
By Request	Biohazard label
By Request	Dry ice shipping label
By Request	Fine Point Markers
By Request	Site and ADCS ID Labels

6.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to kits.iu.edu/T2 to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

Please allow **TWO weeks** for kit orders to be processed and delivered.

7.0 Blood Collection and Processing Procedures

7.1 Labeling Samples

*****Important Note*****

In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly. Draw blood in the following order:

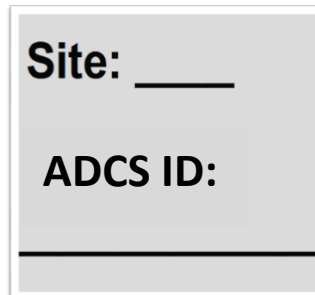
1. Plain Red Top Serum Blood Collection Tube (6 ml)
2. EDTA (Lavender-Top) Blood Collection Tube (6 ml) for PK Analysis
3. EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma

****Label Type Summary****

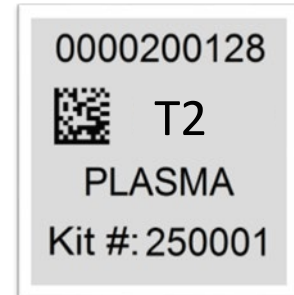
1. Kit Number Label
2. Site and ADCS ID Label
3. Collection and Aliquot Tube Label



The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials. Blood derivatives and CSF collected at the same visit will have **different** Kit Number Labels.



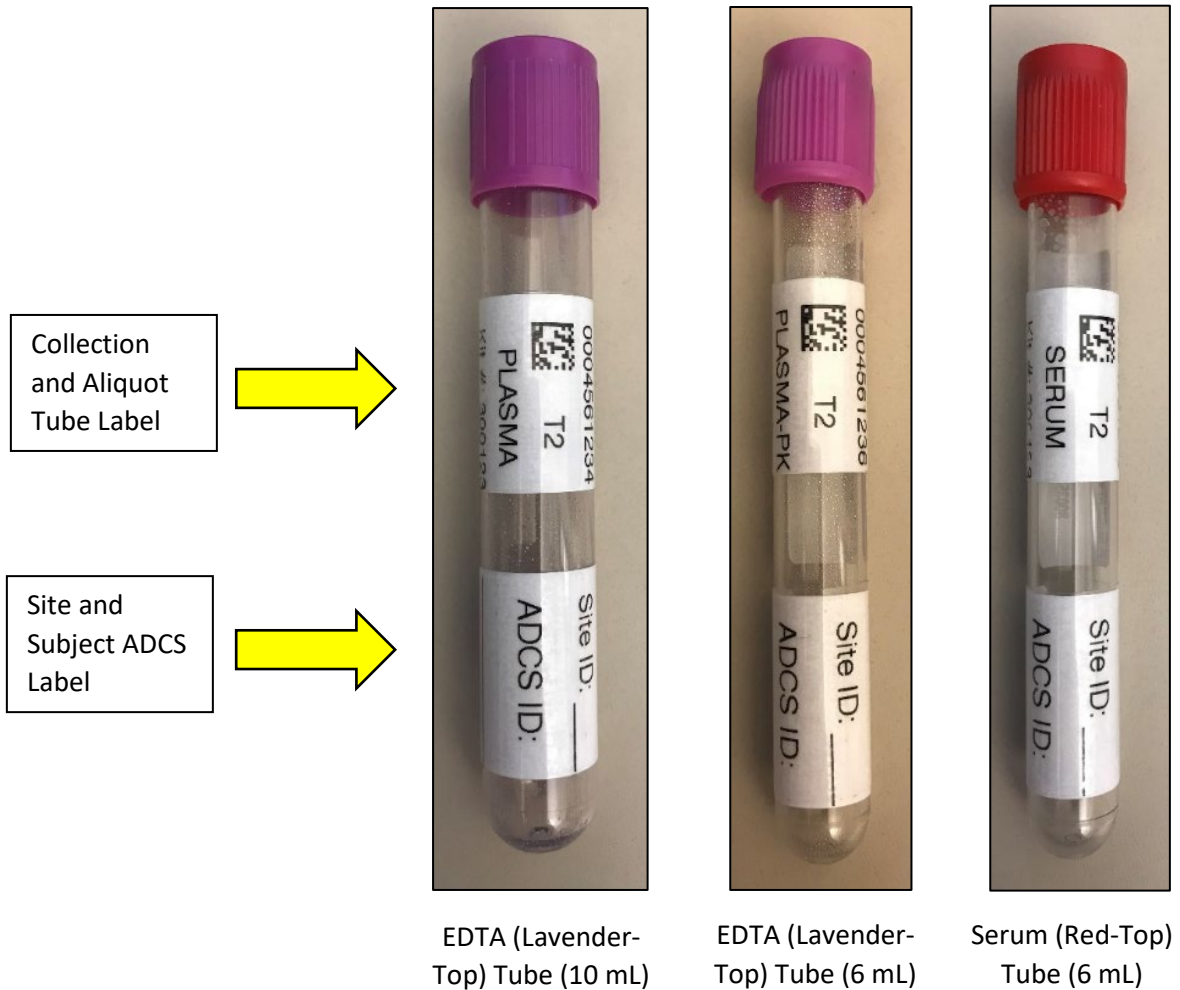
The **Site and ADCS Labels** are placed on all collection tubes, both blood and CSF.



The **Collection and Aliquot Tube Labels** for blood derivatives and CSF are placed on all collection and aliquot tubes.

****Important Note****

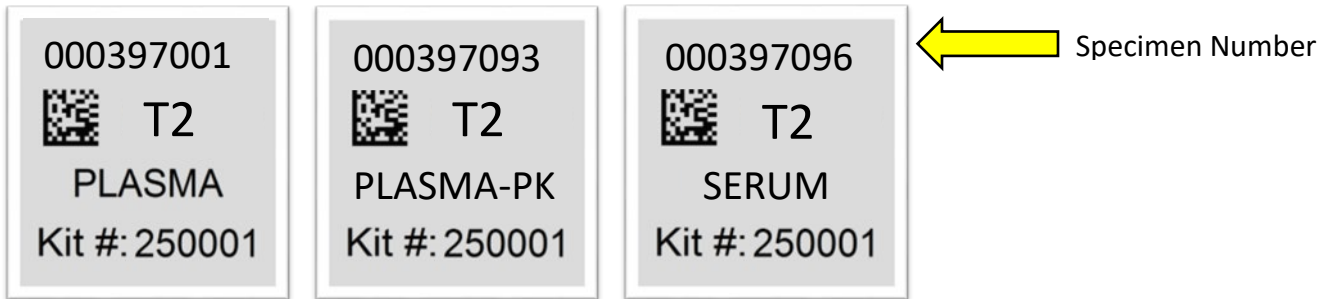
Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the Site and ADCS ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and ADCS label.



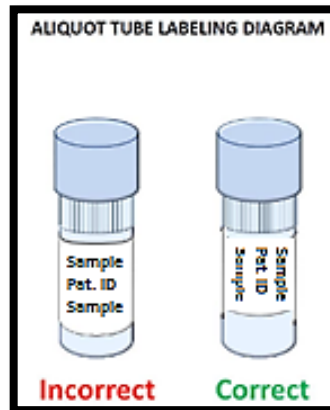
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Place cryovials in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process.



- Using a fine point marker, fill-in and place the Site and ADCS Labels on the collection tubes only (Serum, EDTA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and ADCS ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see labeling diagram below).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



- If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

7.2 Video List

The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: https://ncrad.org/resources_T2_PROTECT_AD.html

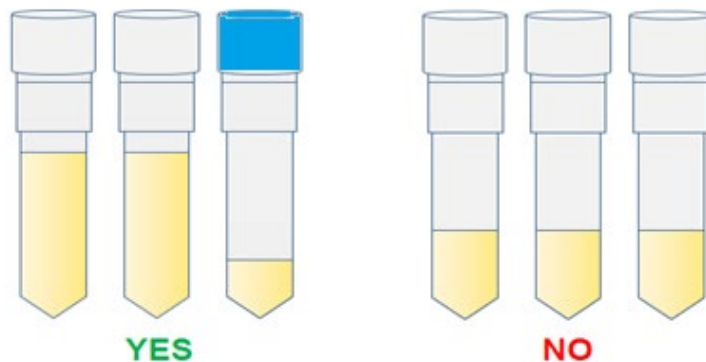
- T2 Protect AD MOP Training
- Plasma and Buffy Coat Processing and Aliquoting
- Serum Processing and Aliquoting
- Frozen Shipping

7.3 Filling Aliquot Tubes (Plasma, Serum, and CSF)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to the assigned volume with the respective biological material after processing is completed (refer to detailed processing instructions for average yield per sample).

Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.

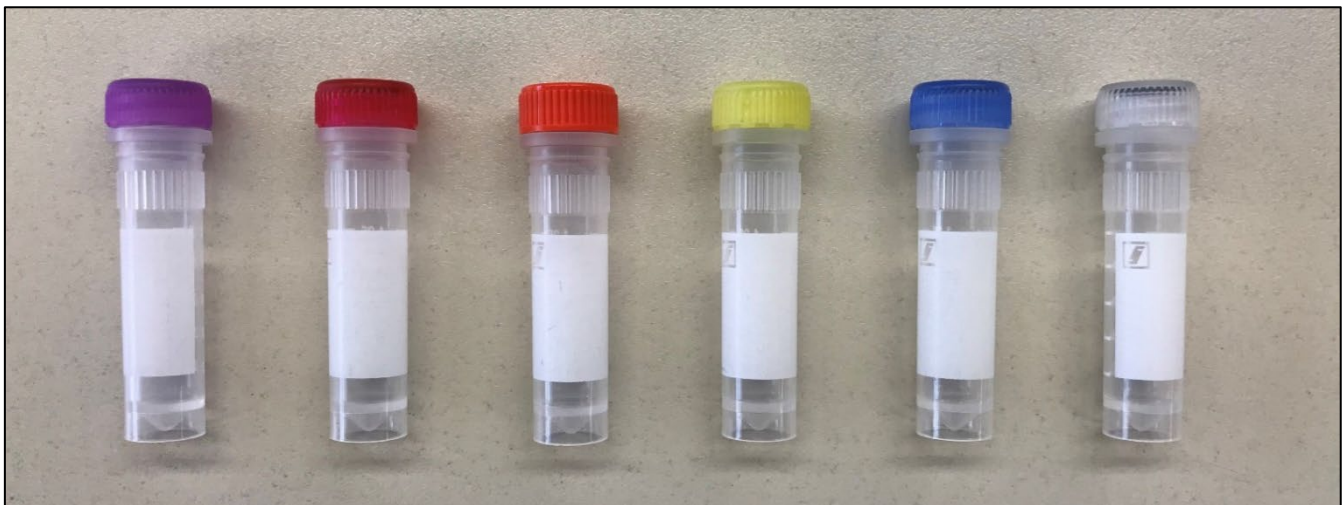
Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.6 ml for plasma and serum. For CSF and plasma PK, fill cryovials with 0.5 ml with any residual will be placed in cryovial with a blue-cap cryovial.



Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml for plasma or serum and anything under 0.5 ml for CSF or plasma PK). Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

To assist in the preparation and aliquoting of samples, colored caps and cap stickers are used for the cryovial tubes. The chart below summarizes the association between cap color and type of cryovial.

Cap Color	Sample Type
Lavender Cap	Plasma/Plasma-PK
Red Cap	Serum
Orange Cap	CSF
Yellow Cap	Local CSF
Blue Cap	Residual
Clear Cap	Buffy Coat



Lavender Top (Plasma)	Red Top (Serum)	Orange Top (CSF)	Yellow Top (Local CSF)	Blue Top (Residual)	Clear Top (Buffy Coat)
-----------------------	-----------------	------------------	------------------------	---------------------	------------------------

7.4 Serum (Red-top) Tube (6 mL) for Serum

Whole Blood Collection for Isolation of Serum: Serum (Red-Top) Tube (6 ml) (for processing of serum aliquots). One Red-Top tube is collected at baseline, week 24, and week 48 visits.

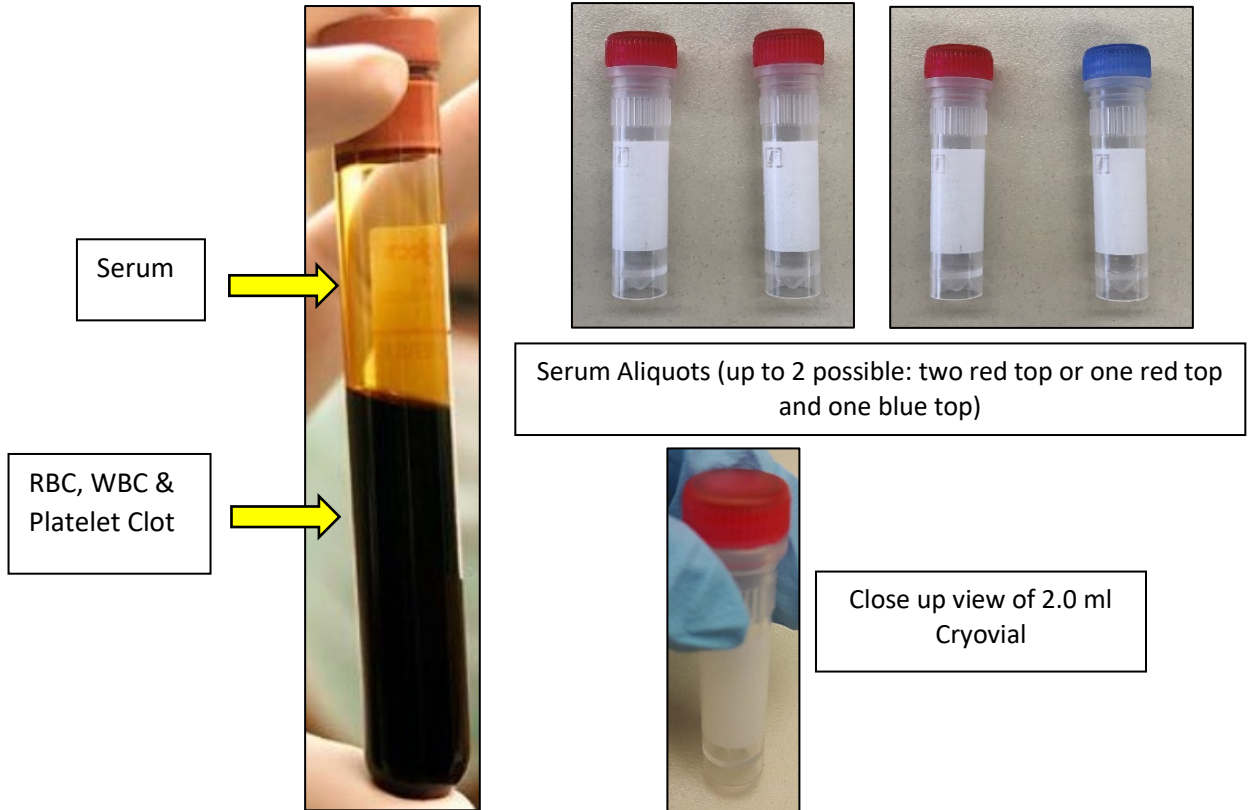
1. Set centrifuge 4°C to pre-chill before use.

2. Place completed Site and ADCS ID Label and Collection and Aliquot **“SERUM”** Tube Labels on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed Collection and Aliquot **“SERUM”** Tube Labels on the (2) 2.0 ml cryovial tubes with red caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual).
3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (6 ml)** using your institution's recommended procedure for standard venipuncture technique

The following techniques shall be used to prevent possible backflow:

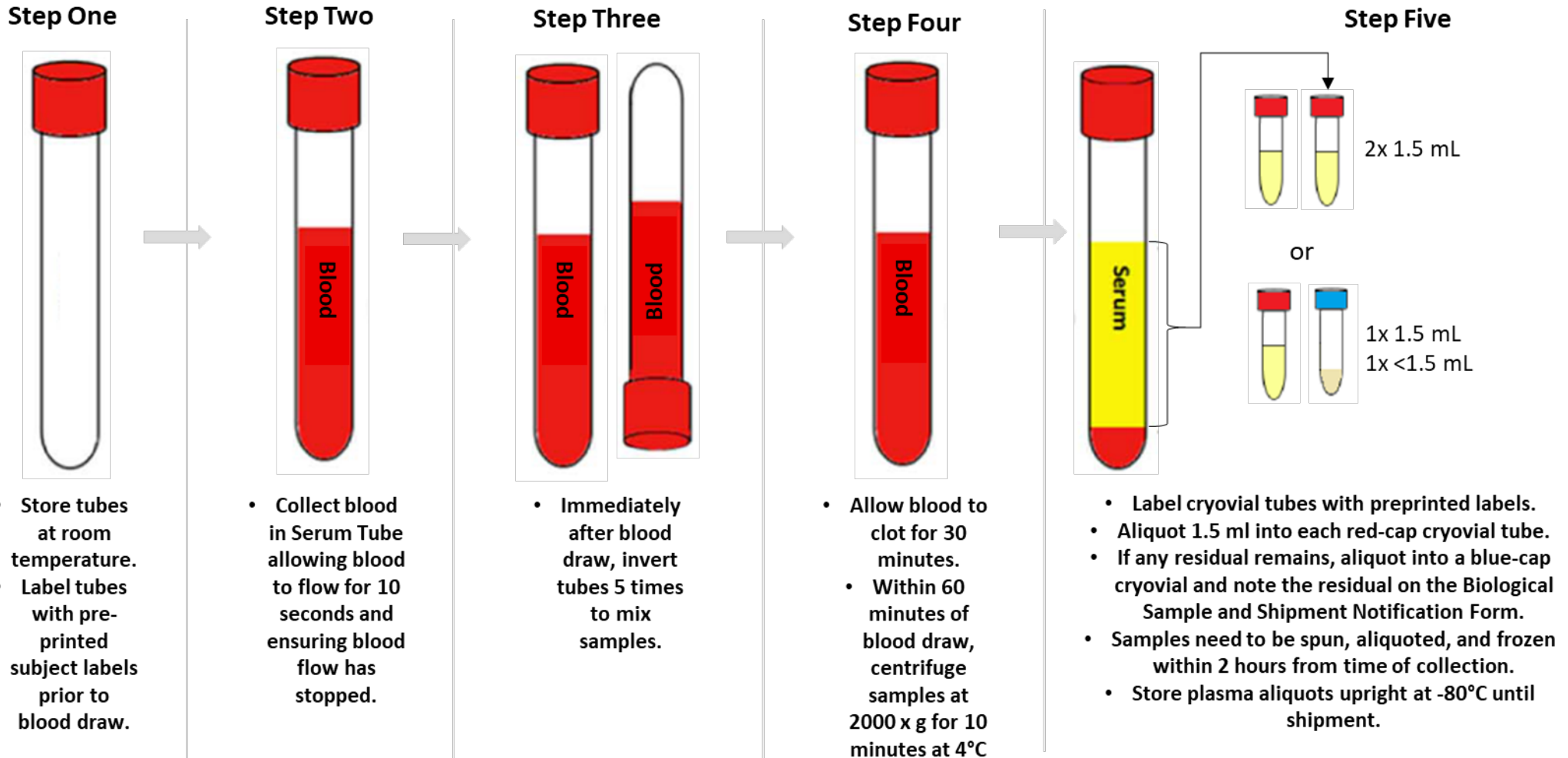
- a. Place donor's arm in a downward position.
 - b. Hold tube in a vertical position, below the donor's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 5 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional Serum tube at this time. Process blood obtained in existing Serum tube.
 5. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**
 6. **CRITICAL STEP: Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. If sample is not clotted allow it to set up to 60 minutes to clot. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.**
 7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in [Appendix A](#) to calculate RPM)**
 - a. Equivalent rpm for spin at 2000 x g
 - b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form [Appendix B](#).

- c. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
 - d. Record time aliquoted on the Biological Sample Shipment and Notification Form
8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable pipette, transfer serum into the pre-labeled cryovials with the red caps. Aliquot 1.5 ml per cryovial (total vials= up to two with 1.5 ml each or one with 1.5 mL and one residual with <1.5 ml). Be sure to only place **serum** in cryovials labeled with the "SERUM" label and red caps. If there is extra serum left, use 1 extra blue-cap cryovial provided for another <1.5 ml aliquot of serum and label as appropriate. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



9. Place the labeled cryovials in a cryobox and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Serum Preparation (6ml Red Top Tube)



7.5 EDTA (Lavender-Top) Blood Collection Tube (6 mL) for Plasma-PK Analysis

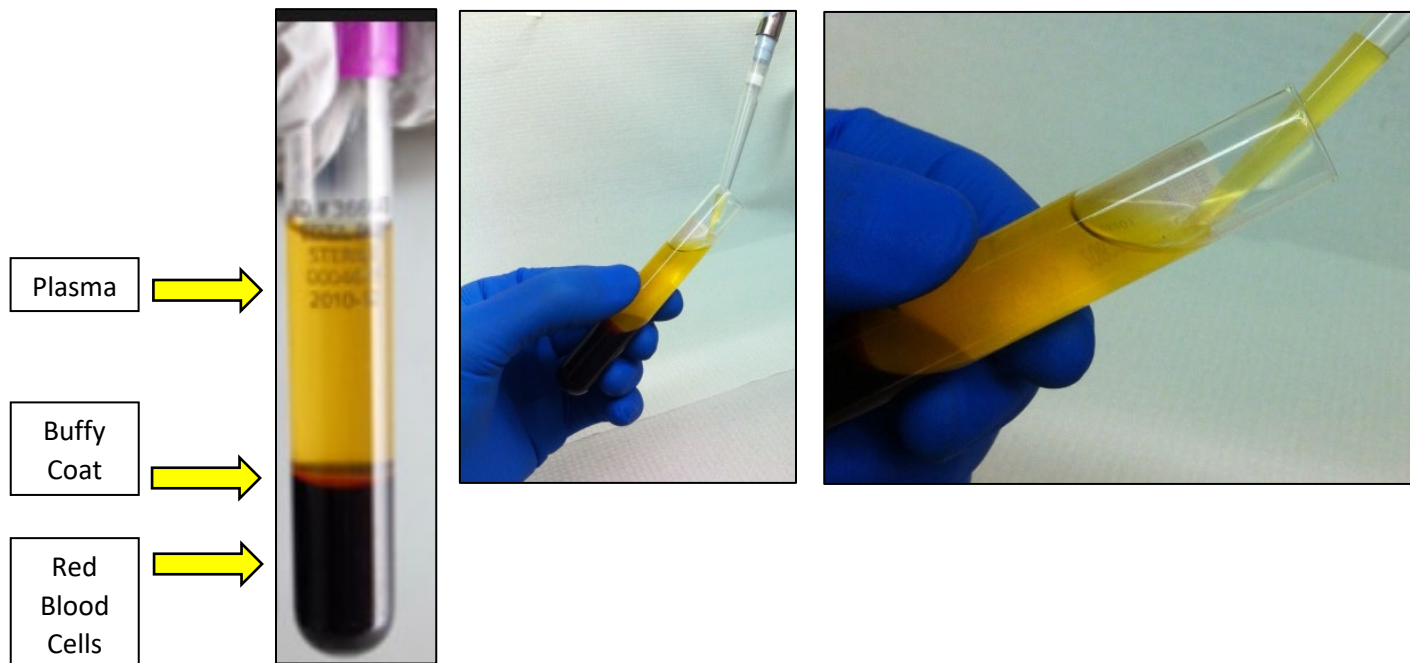
Whole Blood Collection for Plasma-PK Analysis: EDTA (Lavender-Top) Blood Collection Tube (6 ml)

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and ADCS ID Label and pre-printed “**PLASMA-PK**” Collection and Aliquot Tube Label on the lavender-top EDTA tube. Place pre-printed “**PLASMA-PK**” Collection and Aliquot Tube Labels on the six 2.0 ml cryovial tubes with lavender caps and one 2.0 mL cryovial with a blue cap.
3. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process.
4. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tube (6 ml)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
 - b. Hold tube in a vertical position, below the donor's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the ‘Biological Sample and Shipment Notification Form’. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.
 6. **CRITICAL STEP:** Immediately after blood collection, **gently** invert/mix (180 degree turns) the EDTA tube 8-10 times.
 7. **CRITICAL STEP:** Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

- a. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in [Appendix A](#) to calculate RPM.**
 - b. Equivalent rpm for spin at 2000 x g
 - c. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
 - d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
 - e. Record time aliquoted on the Biological Sample and Shipment Notification Form.
8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the collection tube. Transfer plasma into the pre-labeled cryovials. Aliquot 0.5 ml per cryovial (6 vials with 0.5 ml each). Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial with blue cap provided for another <0.5 ml aliquot of plasma. **If a residual aliquot (<0.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



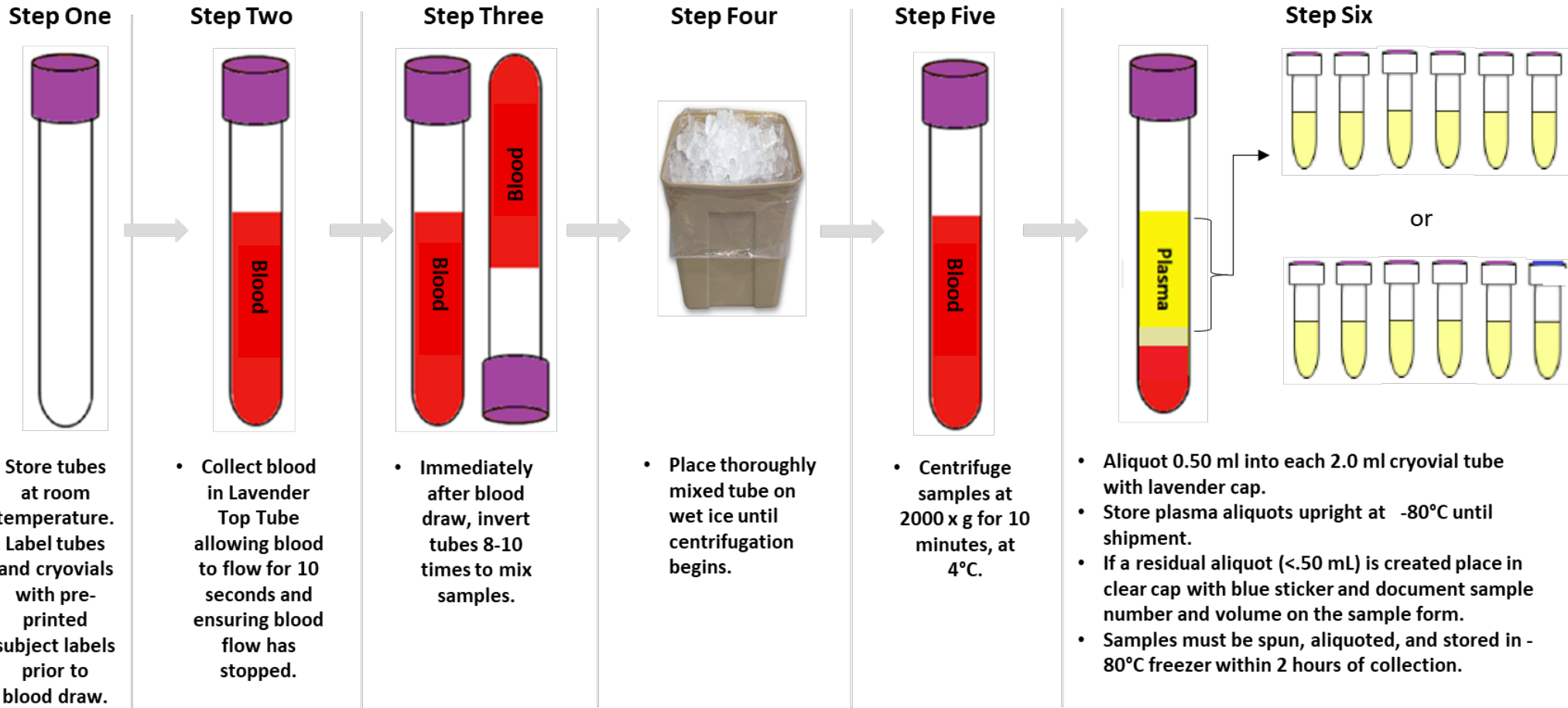
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.



Up to six cryovials possible: five lavender top and one blue top OR six lavender top. Lavender top cryovials have 0.5ml and blue cryovial if <0.5ml remains

9. Place the labeled cryovials in the cryobox and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.
10. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

Plasma-PK (6ml Lavender Top Tube)



7.6 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: EDTA (Lavender-Top) Blood Collection Tube (10 ml) (for processing of plasma aliquots and buffy coat aliquot).

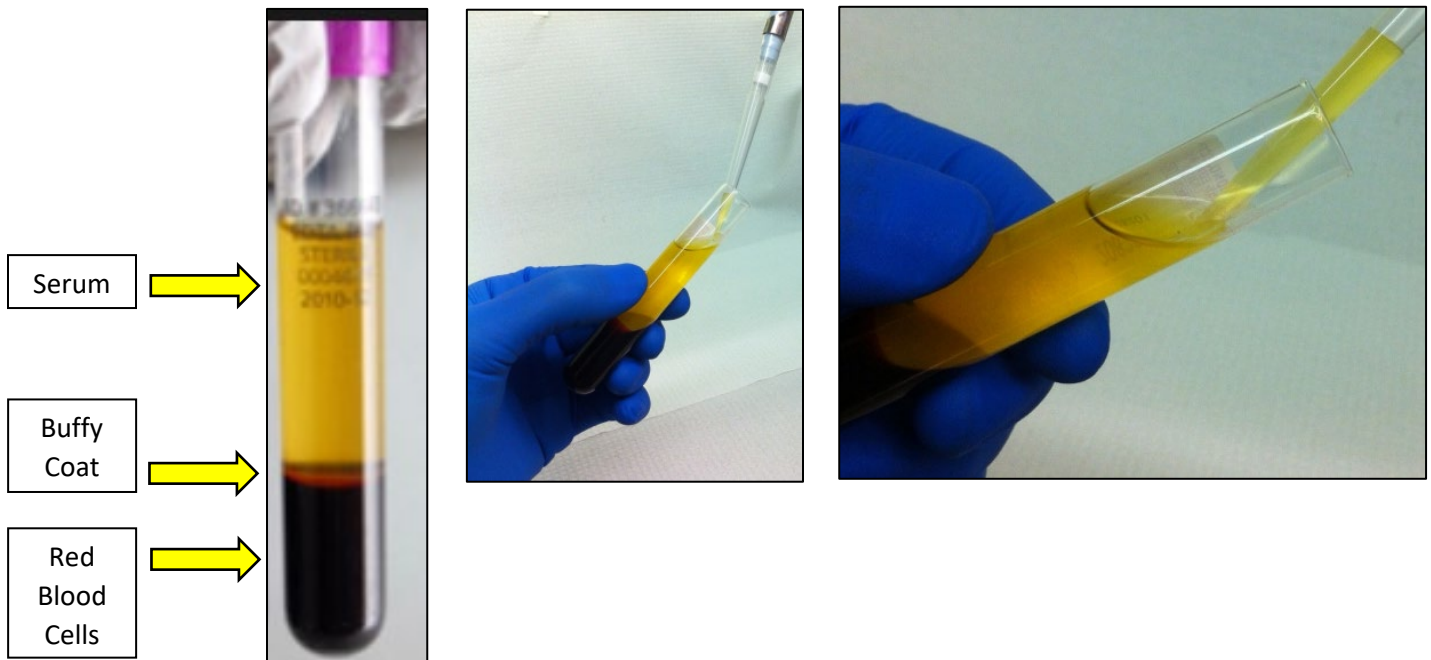
1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and ADCS ID Label and pre-printed “**PLASMA**” Collection and Aliquot Tube Label on the lavender-top EDTA tube. Place pre-printed “**PLASMA**” Collection and Aliquot Tube Labels on the three 2.0 ml cryovial tubes with lavender caps. Place pre-printed “**BUFFY COAT**” Collection and Aliquot Tube Label on the (1) 2 ml cryovial with a clear cap.
3. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process.
4. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
 - b. Hold tube in a vertical position, below the donor's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the ‘Biological Sample and Shipment Notification Form’. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.
 6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.**

7. **CRITICAL STEP: Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.**
 - a. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in [Appendix A](#) to calculate RPM.**
 - b. Equivalent rpm for spin at 2000 x g
 - c. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
 - d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
 - e. Record time aliquoted on the Biological Sample and Shipment Notification Form.

8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the collection tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma into the pre-labeled cryovials. Aliquot 1.5 ml per cryovial (3 vials with 1.5 ml each). Be sure to only place **plasma** in cryovials labeled with “PLASMA” labels. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial with blue cap provided for another <1.5 ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**

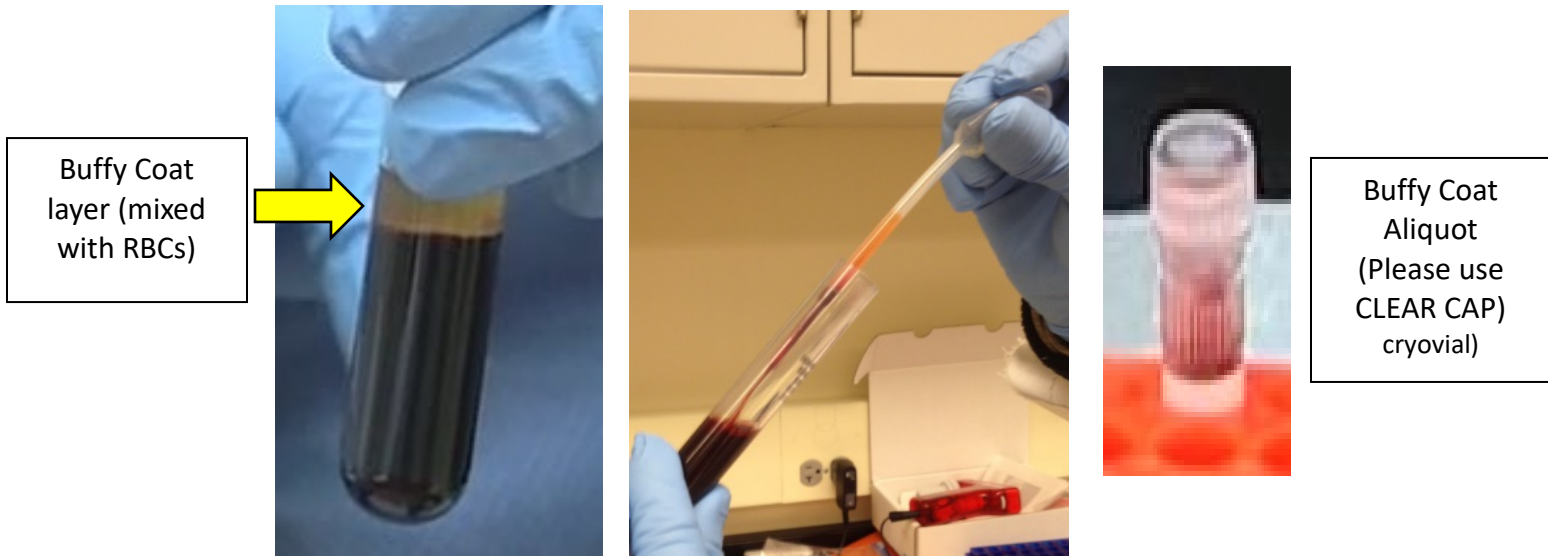


NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.



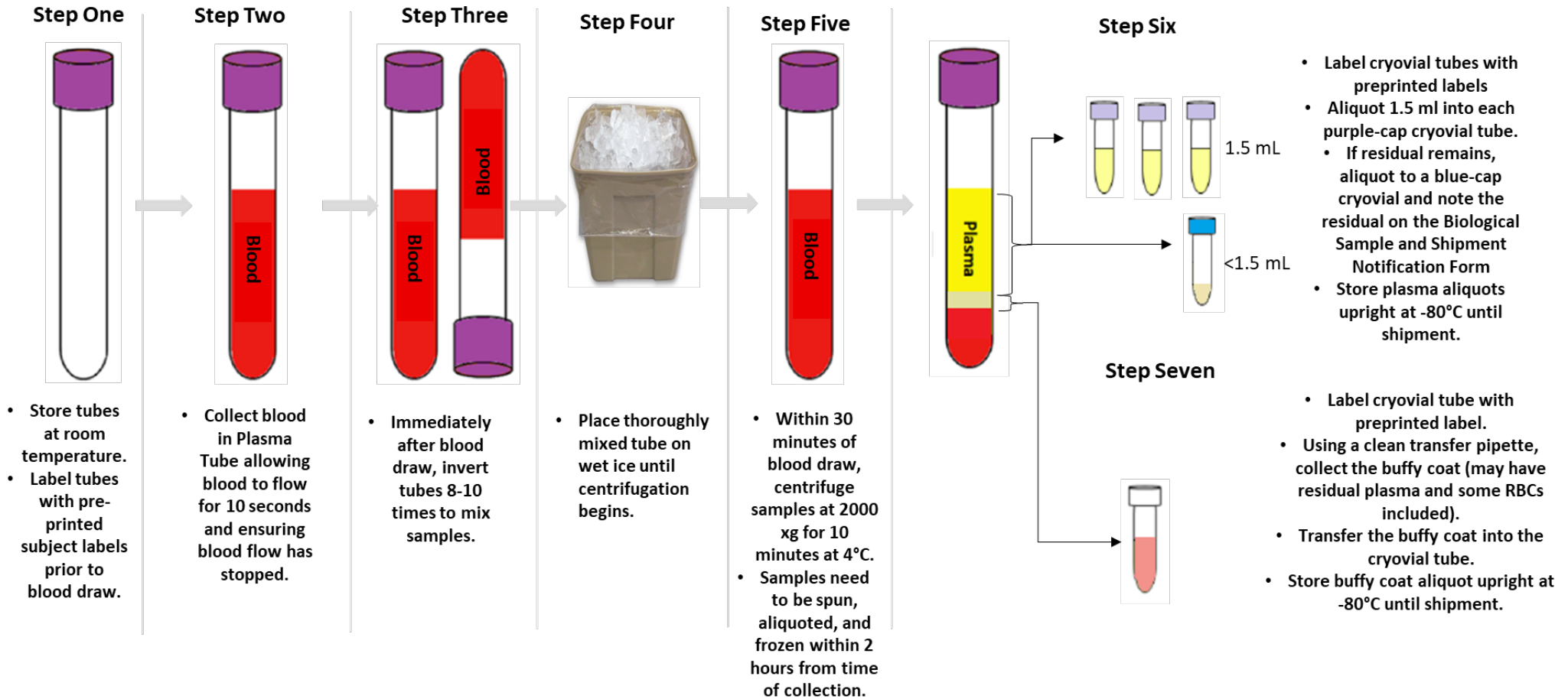
Up to 4 cryovials possible: three lavender top and one blue top. Lavender top cryovials have 1.5ml plasma and blue top cryovial used if <1.5ml remains.

9. Place the labeled cryovials in the cryobox and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.
10. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovial with clear cap using a disposable graduated micropipette. All of the buffy coat will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the clear cap and "BUFFY COAT" label.



11. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
12. Place the labeled cryovial in a cryobox and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

Plasma and Buffy Coat Preparation (10ml Purple Top Tube)



8.0 Cerebrospinal Fluid Collection and Processing Procedures

*****Important Note*****

Fasting prior to the lumbar puncture is not required. However, efforts should be made to ensure each LP done on a participant is performed at the same time of day.

There are general guidelines to follow in regards to CSF Collection.

- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- Do NOT use any extension tubing due to the tendency of manufactured plastic tubing to bind beta amyloid peptides and other important AD biomarkers.
- If LP was attempted but unsuccessful in obtaining CSF, a second attempt under fluoroscopy (if deemed appropriate by site clinician) is allowed.
- LP under fluoroscopy is permitted, if needed. Site personnel should advise the subject that use of fluoroscopy (x-rays) involves exposure to radiation.
- Subjects taking an anti-platelet agent (e.g. aspirin) may, at the discretion of the site clinician, be discontinued from that agent for a period of time prior to lumbar puncture and/or continue off agent for a period of time post LP. Participants who are taking anticoagulants (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) may not undergo an LP and are not suitable to participate in this study.
- Each study participant or a person designated to speak for them will be contacted by phone one day after the LP to confirm participant well-being and to query about any adverse events.
- Identify a physician (e.g., anesthesiologist) able to perform a blood patch for any participant who experiences a post lumbar puncture headache. Find out ahead of time who to call to schedule and perform a blood patch at your center, should the need arise. Ensure billing procedures are in place ahead of time. Ensure you have at least two “Lumbar Puncture Tray Kits” and sufficient “CSF Supplemental Supply Kit” provisions on hand prior to scheduling an LP visit. Also ensure adequate site-provided supplies (see above), including pelleted dry ice. Check expiration dates on all supplies, especially lidocaine.

8.1 Scheduling the LP

LPs can be performed in the morning or in the afternoon. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. CSF amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits.

The LP should be rescheduled if the participant does not feel well or is febrile.

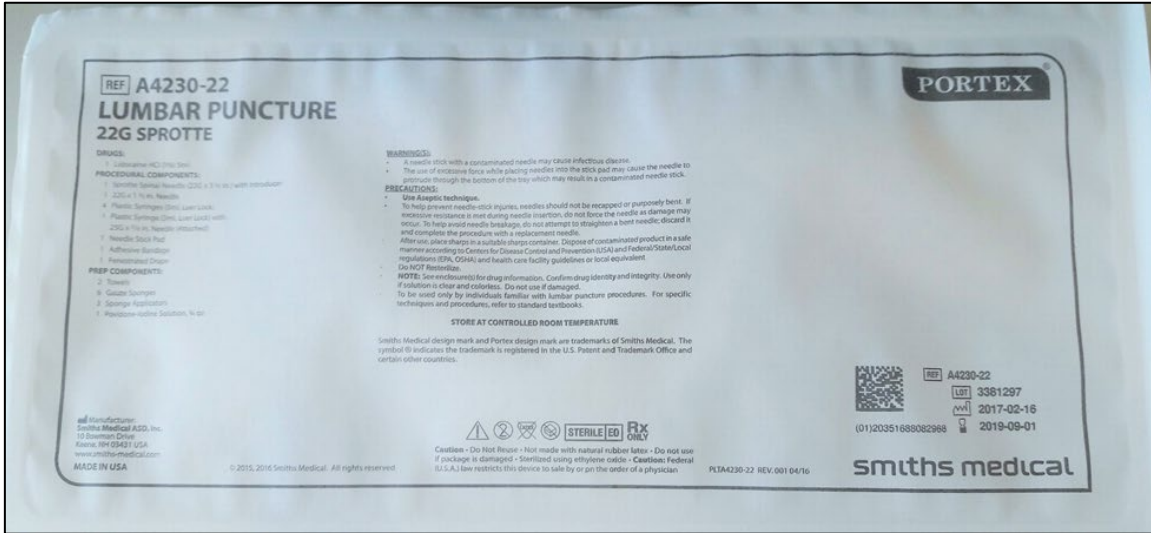
8.2 Performing the LP

The recommended position is sitting. The same position should be used at follow-up LPs. It is critical to try to optimize positioning, and usually requires an assistant. Other positions and needles are allowed (e.g., when using fluoroscopy) but this should be recorded on the CSF Sample and Shipment Notification Form.

On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the lumbar puncture is seated.

Feel the outside of the lumbar puncture kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the lumbar puncture and begin un-wrapping the kit.

Lumbar Puncture Tray Kit Images



Exterior of LP Tray provided by NCRAD which contains the 22 gauge Sprotte Needle with Introducer



Interior of LP Tray Provided by NCRAD

Close up of Sprotte Spinal Needle (22 gauge x 3 ½ in.) with Introducer (24 gauge is equivalent but with lavender top needle)

TOUCH ONLY THE OUTSIDE OF THE PAPER WRAPPER

When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside.

- If you touch any part of the paper wrapper, or if any non-sterile object outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.
- If you are in any doubt as to whether the inside of the wrapper has been touched, throw the kit away and start over.

Cleaning the Lumbar Puncture Site

The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices.

Once the kit is successfully unwrapped, open the bottle of Povidone-Iodine Topical Solution somewhere away from the kit. Use an alcohol swab to remove any loose chunks of dried material off of the bottle top. You don't want anything to fall onto the open and sterile lumbar puncture kit. Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep.

Maintaining the Sterile Field

An important aspect of assisting with a successful lumbar puncture is keeping the field sterile. If there are a number of staff members in the room, please be sure they do not accidentally contaminate the sterile field. Once the person performing the lumbar puncture has donned sterile gloves, additional help may be needed to obtain or un-wrap any new tubes, needles, or supplies.

Unwrapping the Sterile 15 ml Conical Tubes

Note that the 15-ml tubes into which CSF is collected and transferred come individually wrapped and are sterile inside and out. These wrappers should be peeled open by an assistant (not touching the tube) and the tube carefully dropped onto the LP tray or elsewhere in the sterile field in a manner that avoids contamination. Any additional needles or other individually-wrapped sterile items can be handled the same way.

- Do not drop any packaging onto the tray or sterile field.
- Do not let the item touch the outside of the packaging on its way to the tray.

Lidocaine, Syringe with Needle, Gauze Pads

Anesthesia is usually achieved within 2 minutes after injecting the lidocaine. Occasionally, the person performing the lumbar puncture will need to use more

lidocaine to numb up a particular spot, or they may need to move to another spot entirely.

Next, hold the lidocaine bottle upside down and at a slight angle toward the person performing the lumbar puncture so that they can plunge the needle into the bottle and extract some lidocaine without touching you or the bottle. Use two hands to stabilize the bottle. If the person performing the LP requires additional sterile gauze, open the gauze pad the same way as the syringe and needle, by holding open the package so the person performing the lumbar puncture can grab the gauze without touching you or the package.

General CSF Collection Methods

LPs for CSF collection should be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 22 gauge Sprotte needle, although aspiration through this or smaller needles is allowable. Prior approval from the Clinical Core is required before the aspiration method can be utilized. Sites must designate the method of CSF collection for data tracking purpose. It is recommended that CSF be obtained from participants in a sitting position. Alternate needles, positions or methods (e.g., use of fluoroscopy) should be noted on the CSF Sample and Shipment Notification Form.

Collection of CSF by Gravity

After the spinal needle is placed in the intrathecal space and the stylet is withdrawn, CSF should flow freely. **Discard first 1-2mls of CSF if blood tinged. If not blood tinged, collect first 1-2 mLs of CSF into a 15ml conical tube and pipette into the yellow cap cryovial for local lab. Collect another 15 ml CSF into the remaining 15ml conical tube.**

Reminder: If the CSF is blood-tinged, the first 1-2 ml of CSF should be discarded (or more if needed) to clear the blood before collecting the 15-20 ml for CSF analysis. **15 ml is the required MINIMUM for CSF biomarker analysis.** If 15 ml is not obtained and provided to the NCRAD, document the reason for under-collection on the comments section of the CSF Sample and Shipment Notification Form.

Washcloths, Band-Aids, and Clean Up

After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band- Aid should be applied to the puncture site. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

Step by Step Summary of CSF Collection Procedure

Ensure all samples collected are appropriately labeled.

1. Print CSF Sample and Shipment Notification Form.
2. Confirm all supplies, including dry ice (~10 lbs) and wet ice, are available.
3. Label the (15) orange cap cryovials and (1) blue cap cryovial with provided T2 PROTECT AD CSF labels. Do **NOT** open and label the 15-ml tubes that will be kept sterile to collect the CSF.
4. Pre-cool the centrifuge and pre-cool all labeled cryovials on wet ice. Do **NOT** pre-cool the 15-ml tubes that will be kept sterile to collect the CSF.
5. Measure vitals (participant lying down).
6. Record the time of LP and associated information on the CSF Sample and Shipment Notification Form.
7. Collect CSF at the L3/L4 position (or adjacent position) using a 22 gauge Sprotte spinal needle via gravity flow with participant in upright position (or document alternate method on CSF Sample and Shipment Notification Form) following these steps:
 - a. Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) using the 15ml conical tube. If not bloody, transfer first 1-2ml into yellow cap cryovial for local lab.
 - b. Collect an additional 15 ml CSF into the **UNLABELED-STERILE** 15-ml polypropylene tubes from the "CSF Supply Kit". 15 ml is the required **MINIMUM**.
 - c. If using aspiration, use **ONLY** the polypropylene syringes included in the "Lumbar Puncture Collection Kit" and transfer **DIRECTLY** into the **UNLABELED-STERILE** 15-ml polypropylene tube from the "CSF Supply Kit". There are four 6 ml Luer lock polypropylene syringes in the "Lumbar Puncture Collection Kit." Note this on the CSF Sample and Shipment Notification Form.
8. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:
 - a. Place samples upright on wet ice prior to processing. Within 15 minutes of collection, centrifuge briefly at low speed (2000 x g, 10 min, 4°C with brake turned off) to pellet any cellular debris.
 - b. Aliquot 0.5ml into the orange-cap cryovials. If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample and Shipment Notification Form.
 - c. Store CSF aliquots at -80°C and record time of freezing on CSF Sample and Shipment Notification Form.



10. Provide food and drink to participant (participant may lay flat to minimize the chance of a post-LP headache).
11. Measure vital signs again one hour post-LP.
12. If vital signs are stable and participant feels OK one hour post-procedure, participant may sit upright, stand, and walk.
13. Enter collection data into the EDC website on day of visit.

CSF Preparation

Step One



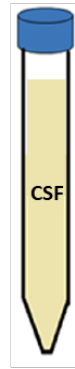
- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two



- Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) into 15 ml conical tube.
- If not bloody, transfer 1-2 ml into the yellow-cap cryovial.
- Send to local lab for testing.

Step Three



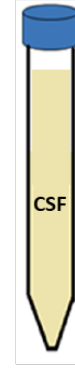
- Collect another 15 ml CSF into a new 15 ml sterile conical tube.

Step Four



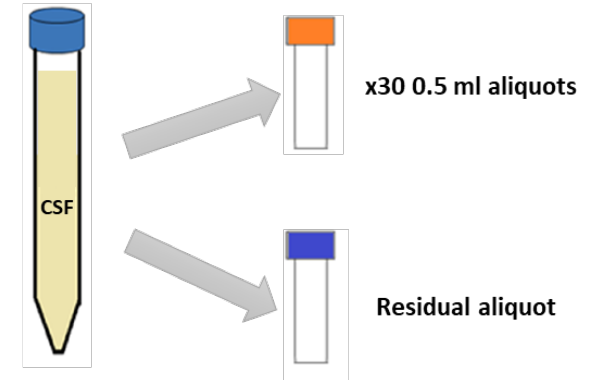
- Place sample upright on wet ice until centrifugation begins.

Step Five



- Within 15 minutes of collection, centrifuge sample at 4°C for 10 minutes (no brake).

Step Six



- Using a clean transfer pipette, aliquot 0.5 ml into the orange-cap cryovials, leaving debris at the bottom of the conical tube.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
- Store CSF aliquots at -80°C until shipment.

LUMBAR PUNCTURE FOLLOW-UP PHONE CALL

This should be done the day after the lumbar puncture for all participants who had the procedure.

SUGGESTED MANAGEMENT OF POST-LUMBAR PUNCTURE HEADACHE

Classic post-lumbar puncture (low pressure) headache is worse when the participant is upright (sits or stands) and improves when the participant is recumbent with the head **no higher** than the spinal cord.

Safety and comfort of the T2 PROTECT AD LP is maximized by the use of atraumatic needles. The T2 PROTECT AD protocol requires use of a 22 gauge Sprotte needle. Lumbar puncture is a standard procedure for collection of CSF but may be associated with pain during the performance of the procedure, comparable to the level of pain experienced during a blood draw. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after lumbar puncture, probably due to leakage of CSF. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed.

Prevention: Use of a small and atraumatic needle with careful technique are helpful in preventing lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) for 24 hours after the LP may minimize the chance of a lumbar puncture headache.

Treatment of headache after a lumbar puncture:

- Limit physical activity as much as possible for at least 24 hours post-procedure.
- Increase oral fluid intake. Caffeine may be helpful.
- Routine analgesics such as acetaminophen may be used.

Post-lumbar puncture headache often resolves with the above treatment. If the headache persists after 24 hours of this management, it will likely require a blood patch. A blood patch *typically* relieves the headache instantly.

Prior approval from the T2 PROTECT AD Coordinating Center is not necessary to perform a blood patch. However, depending on the site, local IRB approval may be required. Sites will be responsible for costs related to the performance of a blood patch.

9.0 Sample Redraws

*****Important Note*****

If challenges arise during the blood draw process, it is advised that the phlebotomist discontinue the draw. Attempt to process and submit any blood-based specimens that have already been collected to NCRAD.

Redraws will be scheduled for samples submitted to NCRAD.

There may be situations that arise that require a patient sample to be redrawn from certain cycles/visits. At those times, NCRAD study staff will alert site coordinators that a participant sample has failed and should be redrawn. This can happen for several reasons, including insufficient blood at the time the sample was drawn, temperature storage extremes, or even shipping errors.

1. If the biospecimens at a scheduled visit **are partially** collected:
 - a. Attempt to process and submit any samples that were able to be collected during the visit.
 - b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
 - i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
 - ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
 - c. Contact a NCRAD coordinator and alert them of the challenging blood draw.

2. If the biospecimens at a scheduled visit **are not** collected:
 - a. Contact the T2 PROTECT AD Project Manager and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.

Schedule participant for a re-draw visit as quickly as possible.

10.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, please contact NCRAD with questions and information regarding resources.

Sample Type	Processing/Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA tube) for plasma-pk	0.5 ml aliquots per 2.0 ml cryovials	Up to 6	Frozen
Whole blood (Lavender-Top EDTA tube) for isolation of plasma and buffy coat	1.5 ml plasma aliquots per 2.0 ml cryovials	Up to 4	Frozen
	0.75 ml buffy coat aliquot per 2.0 ml cryovial	1	Frozen
Whole blood (Red-Top tube) for isolation of serum	1.5 ml serum aliquots per 2.0 ml cryovials	Up to 2	Frozen
CSF	0.5 ml CSF aliquots per 2.0 ml cryovials	Up to 30	Frozen

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

10.1 Frozen Batch Shipping Instructions

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED
MONDAY-WEDNESDAY ONLY!

*** Packing and Labeling Guidelines ***

- The primary receptacle (frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (within the cryovial box containing the frozen cryovials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
 - ✓ Sender's name and address
 - ✓ Recipient's name and address
 - ✓ Responsible Person
 - ✓ The words "Biological Substance, Category B"
 - ✓ UN3373
 - ✓ Class 9 label including UN 1845, and net weight of dry ice contained



1. A sample shipment to NCRAD should be initiated when a study site has five (5) cryoboxes of samples or every three (3) months, whichever is sooner.
2. Contact FedEx to confirm service is available and schedule package to be picked up.
3. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the Completed Biological Sample and Shipment Form to the email ([Appendix B](#)). If email is unavailable, please call NCRAD and do not ship until you have notified NCRAD coordinators of the shipment in advance.

4. Place all labeled and frozen plasma, serum, and buffy coat aliquots in a cryobox.
5. If collecting CSF, place up to 25 CSF aliquots in a 25-slot cryobox and the remaining aliquots in a second cryobox (can be combined with plasma, serum, and buffy coat aliquots). Label the outside of the cryobox with the kit number label and place in a clear biohazard bag. Do not remove absorbent material found in the bag and seal according to the instructions on the bag.



6. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
7. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. Layer dry ice and cryoboxes as necessary.
8. The inner Styrofoam shipping container must contain approximately 30-45 lbs (or ~21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with
Batched Samples and Dry Ice



9. Replace the lid of the Styrofoam container. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape. Be sure to NOT completely seal the outer cardboard box with tape, as the dry ice needs to vent.
10. Complete the FedEx return airbill with the following information:
 - Section 1, “From”: fill in your name, address, phone number, and Site FedEx Account Number.
 - Section 2, “Your Internal Billing Reference”: add any additional information required by your site.
 - Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of dry ice in kg.
 - Section 7, “Payment”, check third party and bill transportation costs to the T2 PROTECT AD study FedEx account number.
11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
 - Your name and return address
 - Net weight of dry ice in kg (must match amount on the airbill)
 - Consignee name and address:

NCRAD
IU School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
 - Do not cover any part of this label with other stickers, including pre-printed address labels.
12. Apply all provided warning labels and the completed FedEx return airbill to the outside of the package, taking care not to overlap labels.

13. Hold packaged samples in -80C freezer until time of FedEx pick-up/drop-off.
14. Specimens should be sent to the address below via FedEx Priority Overnight. Frozen specimens should be sent **Monday through Wednesday** to avoid any potential shipping delays. FedEx does not replenish dry ice if shipments are delayed or held over the weekend.

NCRAD

IU School of Medicine

351 W. 10th St. TK-217

Indianapolis, IN 46202

Phone: 1-800-526-2839

15. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the FedEx tracking number in your email.

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY
SHIPMENT/DELIVERY OF SAMPLES!

Remember to complete the Biological Sample and Shipment Notification (Appendix B), include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include Fed Ex tracking number in email) IN ADVANCE to confirm the shipment.

In addition to tracking and reconciliation of samples, the condition and number of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

11.0 Data Queries and Sample Reconciliation

The Laboratory worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Missing samples

- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the ADCS database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological or CSF Sample and Shipment Notification Form

12.0 Appendices List

Appendix A. Rate of Centrifuge Worksheet

Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you.

Submitter Information

Name:

Site:

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor:

Swing Bucket Rotor:

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Calculating RPM from G-Force:

$$RCF = \left(\frac{RPM}{1,000} \right)^2 \times r \times 1.118 \Rightarrow RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1,000$$

RCF = Relative Centrifugal Force (G-Force)

RPM = Rotational Speed (revolutions per minute)



R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax)

alzstudy@iu.edu



Appendix B. Biological Sample and Shipment Notification Form

Biological Sample and Shipment Notification Form

Please email or fax the form on or prior to the date of shipment.

To: Kelley Faber Email: alzstudy@iu.edu FAX: 317-321-2003 Phone: 1-800-526-2839			
<i>General Information:</i>		FedEx tracking #: _____	
From: _____	Date: _____		
Phone: _____	Email: _____		
Study: T2 PROTECT AD	GUID: _____	Kit #:	KIT BARCODE
Visit: Baseline	Week: 4 8 12 24 48		
Site ID: _____	ADCS IND #: _____		
Sex: M F	Year of Birth: _____	CSF Collected?	Yes No
<i>Blood Collection:</i>			
1. Date Drawn: _____ [MMDDYY]	2. Time of Draw: _____ [HHMM]		
3. Last time subject ate: _____ [MMDDYY]	4. Last time subject ate: _____ [HHMM]		
<i>Blood Processing:</i>			
Serum (Red-top) Tube (6 mL)		Plasma & Buffy Coat (Lavender-top) Tube (10 mL)	
Time spin started: _____ [HHMM]	Duration of centrifuge: _____ Minutes	Temp of Centrifuge: _____ °C	Rate of centrifuge: _____ x g
Original volume drawn (1 x 6 mL tube): _____ mL	Time aliquoted: _____ [HHMM]	Original volume drawn (1 x 10 mL tube): _____ mL	Time aliquoted: _____ [HHMM]
Number of 1.5 mL serum aliquots created (red cap): _____	If applicable, volume of residual serum aliquot (less than 1.5 mL in blue cap): _____ mL	Number of 1.5 mL plasma aliquots created (lavender cap): _____	If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap): _____ mL
If applicable, specimen number of residual serum aliquot (last four digits): _____	Time aliquots placed in freezer: _____ [HHMM]	If applicable, specimen number of residual plasma aliquot (last four digits): _____	Time aliquots placed in freezer: _____ [HHMM]
Storage temperature in freezer: _____ °C		Storage temperature in freezer: _____ °C	
		Buffy coat aliquot created (clear cap, one per 10 mL EDTA tube)	_____ mL
Plasma-PK (Lavender-top) Tube (6 ml)			
Time spin started: _____ [HHMM]	Duration of centrifuge: _____ Minutes	Original volume drawn (1 x 6 mL tube): _____ mL	
Temp of Centrifuge: _____ °C	Rate of centrifuge: _____ x g	Number of 0.5 mL plasma-pk aliquots: _____	
Time aliquoted: _____ [HHMM]		If applicable, volume of residual plasma-pk aliquot (blue cap) & last 4 digits: _____	
		Time aliquots placed in freezer: _____ [HHMM]	
		Storage temperature in freezer: _____ °C	
<i>Notes:</i>			



Appendix C. CSF Sample and Shipment Notification Form

CSF Sample and Shipment Notification Form

Please email or fax the form on or prior to the date of shipment.

To: Kelley Faber Email: alzstudy@iu.edu FAX: 317-321-2003 Phone: 1-800-526-2839			
General Information:		FedEx tracking #: _____	
From: _____	Date: _____		
Phone: _____	Email: _____		
Study: T2 PROTECT AD	GUID: _____	Kit #:	KIT BARCODE
Visit: Screening	Week 24	Week 48	
Site ID: _____	ADCS IND #: _____	Gauge needle used for LP:	22G 24G
Sex: M F	Year of Birth: _____	CSF Collected?	Yes No
CSF Collection:			
5. Date of collection:	[MMDDYY]	6. Time of collection:	[HHMM]
7. Last time subject ate:	[MMDDYY]	8. Last time subject ate:	[HHMM]
9. Collection process:	Gravity Method	Aspiration	
CSF Processing:			
Time spin started:	_____ [HHMM]		
Duration of centrifuge:	_____ Minutes		
Temp of Centrifuge: _____ °C	Rate of centrifuge: _____ x g		
Total amount of CSF collected:	_____ mL		
Time aliquoted:	_____ [HHMM]		
Number of 0.5 mL CSF aliquots created (orange cap):	_____ x 0.5 mL		
If applicable, volume of residual CSF aliquot (less than 0.5 mL in blue cap):	_____ mL		
If applicable, specimen number of residual serum aliquot (last four digits):	_____		
Time frozen:	_____ [HHMM]		
Storage temperature in freezer:	_____ °C		
Notes:			



Appendix D. GUID Demographics Form

Please be certain to collect the following demographic information to generate a Global Unique Identifier:

1. Complete legal given (first) name of subject at birth: _____
2. Complete additional (middle) name or names at birth: _____
3. Complete legal family (last) name of subject at birth: _____
4. Suffix: _____
5. Date of Birth: _____
6. Name of city/municipality in which subject was born: _____
7. Country of birth: _____